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* * * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 16:37:59 ON 09 JAN 2008

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 16:38:14 ON 09 JAN 2008
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s chaperon? or aggregat?(5a)(inhibit? or suppress? or prevent? or decreas?)
FILE 'MEDLINE'
         18469 CHAPERON?
        130845 AGGREGAT?
       1390221 INHIBIT?
        324425 SUPPRESS?
       1132552 PREVENT?
       1134113 DECREAS?
         28768 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L1       46384 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'SCISEARCH'
         19109 CHAPERON?
        169663 AGGREGAT?
       1177864 INHIBIT?
        352434 SUPPRESS?
        496182 PREVENT?
       1198877 DECREAS?
         14577 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L2       32757 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'LIFESCI'
          6502 CHAPERON?
         33673 AGGREGAT?
        387405 INHIBIT?
        106555 SUPPRESS?
        108220 PREVENT?
        279513 DECREAS?
          3067 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L3        9211 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'BIOTECHDS'
           872 CHAPERON?
          4794 AGGREGAT?
         67214 INHIBIT?
         12229 SUPPRESS?
         33717 PREVENT?
         30017 DECREAS?
           719 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L4        1555 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'BIOSIS'
         15922 CHAPERON?
        142346 AGGREGAT?
       1582074 INHIBIT?
        351286 SUPPRESS?
```

```
        550985 PREVENT?
       1354548 DECREAS?
         18518 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L5       33580 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'EMBASE'
         13870 CHAPERON?
        108677 AGGREGAT?
       1279902 INHIBIT?
        299727 SUPPRESS?
        878779 PREVENT?
       1056443 DECREAS?
         18016 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L6       31123 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'HCAPLUS'
         18675 CHAPERON?
        250264 AGGREGAT?
       1993587 INHIBIT?
        440923 SUPPRESS?
        996586 PREVENT?
       2453976 DECREAS?
         34414 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L7       52083 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'NTIS'
           117 CHAPERON?
         13507 AGGREGAT?
         22511 INHIBIT?
         15448 SUPPRESS?
         54431 PREVENT?
         54034 DECREAS?
           172 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L8           286 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'ESBIOBASE'
         11228 CHAPERON?
         44845 AGGREGAT?
        542878 INHIBIT?
        146577 SUPPRESS?
        180476 PREVENT?
        449169 DECREAS?
          5712 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L9       16269 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'BIOTECHNO'
          5718 CHAPERON?
         22679 AGGREGAT?
        301415 INHIBIT?
         79558 SUPPRESS?
         71195 PREVENT?
        171676 DECREAS?
          3551 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L10       8911 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

FILE 'WPIDS'
           643 CHAPERON?
         68414 AGGREGAT?
        286804 INHIBIT?
```

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        266194 SUPPRESS?
       1903331 PREVENT?
        286510 DECREAS?
          8142 AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR DECREAS?)
L11       8754 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

TOTAL FOR ALL FILES
L12     240913 CHAPERON? OR AGGREGAT?(5A)(INHIBIT? OR SUPPRESS? OR PREVENT? OR
               DECREAS?)

=> s l12(10a)fusion
FILE 'MEDLINE'
        153107 FUSION
L13        109 L1 (10A)FUSION

FILE 'SCISEARCH'
        139607 FUSION
L14        109 L2 (10A)FUSION

FILE 'LIFESCI'
         43845 FUSION
L15         72 L3 (10A)FUSION

FILE 'BIOTECHDS'
         27435 FUSION
L16         70 L4 (10A)FUSION

FILE 'BIOSIS'
        111256 FUSION
L17        114 L5 (10A)FUSION

FILE 'EMBASE'
         89782 FUSION
L18         97 L6 (10A)FUSION

FILE 'HCAPLUS'
        280236 FUSION
L19        292 L7 (10A)FUSION

FILE 'NTIS'
         23224 FUSION
L20          0 L8 (10A)FUSION

FILE 'ESBIOBASE'
         47019 FUSION
L21         89 L9 (10A)FUSION

FILE 'BIOTECHNO'
         42345 FUSION
L22         61 L10(10A)FUSION

FILE 'WPIDS'
         56908 FUSION
L23         73 L11(10A)FUSION

TOTAL FOR ALL FILES
L24       1086 L12(10A) FUSION


=> s l12(10a)(archae? or methanococcus or thermococcus or methanosarcina)
FILE 'MEDLINE'
         13255 ARCHAE?
          1058 METHANOCOCCUS
           451 THERMOCOCCUS
```

```
           785 METHANOSARCINA
L25        132 L1 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'SCISEARCH'
         23833 ARCHAE?
          1391 METHANOCOCCUS
           671 THERMOCOCCUS
          1449 METHANOSARCINA
L26        158 L2 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'LIFESCI'
          8345 ARCHAE?
           829 METHANOCOCCUS
           346 THERMOCOCCUS
           849 METHANOSARCINA
L27         93 L3 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'BIOTECHDS'
          1773 ARCHAE?
           241 METHANOCOCCUS
           245 THERMOCOCCUS
           426 METHANOSARCINA
L28         31 L4 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'BIOSIS'
         31751 ARCHAE?
          1632 METHANOCOCCUS
           632 THERMOCOCCUS
          1614 METHANOSARCINA
L29        145 L5 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'EMBASE'
          9952 ARCHAE?
          1017 METHANOCOCCUS
           415 THERMOCOCCUS
           948 METHANOSARCINA
L30        116 L6 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'HCAPLUS'
         27262 ARCHAE?
          1612 METHANOCOCCUS
           826 THERMOCOCCUS
          1608 METHANOSARCINA
L31        228 L7 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'NTIS'
          4801 ARCHAE?
            37 METHANOCOCCUS
             3 THERMOCOCCUS
            49 METHANOSARCINA
L32          4 L8 (10A) (ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
               INA)

FILE 'ESBIOBASE'
         10844 ARCHAE?
           674 METHANOCOCCUS
           375 THERMOCOCCUS
           575 METHANOSARCINA
```

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L33          118 L9 (10A)(ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
                 INA)

FILE 'BIOTECHNO'
            5361 ARCHAE?
             719 METHANOCOCCUS
             284 THERMOCOCCUS
             662 METHANOSARCINA
L34           78 L10(10A)(ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
                 INA)

FILE 'WPIDS'
             707 ARCHAE?
             108 METHANOCOCCUS
             162 THERMOCOCCUS
              87 METHANOSARCINA
L35           23 L11(10A)(ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
                 INA)

TOTAL FOR ALL FILES
L36         1126 L12(10A)(ARCHAE? OR METHANOCOCCUS OR THERMOCOCCUS OR METHANOSARC
                 INA)

=> s l24 and l36
FILE 'MEDLINE'
L37             1 L13 AND L25

FILE 'SCISEARCH'
L38             1 L14 AND L26

FILE 'LIFESCI'
L39             0 L15 AND L27

FILE 'BIOTECHDS'
L40             4 L16 AND L28

FILE 'BIOSIS'
L41             1 L17 AND L29

FILE 'EMBASE'
L42             1 L18 AND L30

FILE 'HCAPLUS'
L43            14 L19 AND L31

FILE 'NTIS'
L44             0 L20 AND L32

FILE 'ESBIOBASE'
L45             1 L21 AND L33

FILE 'BIOTECHNO'
L46             0 L22 AND L34

FILE 'WPIDS'
L47             4 L23 AND L35

TOTAL FOR ALL FILES
L48            27 L24 AND L36

=> s peptidyl prolyl(3w)isomerase# or ppiase# or ppi
FILE 'MEDLINE'
            14055 PEPTIDYL
             6254 PROLYL
              888 PEPTIDYL PROLYL
```

```
                (PEPTIDYL(W)PROLYL)
          15290 ISOMERASE#
            820 PEPTIDYL PROLYL(3W)ISOMERASE#
            391 PPIASE#
           4179 PPI
L49        5076 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

FILE 'SCISEARCH'
           4536 PEPTIDYL
           5921 PROLYL
           1401 PEPTIDYL PROLYL
                  (PEPTIDYL(W)PROLYL)
          12681 ISOMERASE#
           1340 PEPTIDYL PROLYL(3W)ISOMERASE#
            395 PPIASE#
           4586 PPI
L50        6003 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

FILE 'LIFESCI'
           2234 "PEPTIDYL"
           1708 "PROLYL"
            482 PEPTIDYL PROLYL
                  ("PEPTIDYL"(W)"PROLYL")
           4527 ISOMERASE#
            458 PEPTIDYL PROLYL(3W)ISOMERASE#
            208 PPIASE#
            798 PPI
L51        1289 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

FILE 'BIOTECHDS'
            291 PEPTIDYL
            344 PROLYL
             82 PEPTIDYL PROLYL
                  (PEPTIDYL(W)PROLYL)
           2476 ISOMERASE#
             78 PEPTIDYL PROLYL(3W)ISOMERASE#
             36 PPIASE#
            184 PPI
L52         269 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

FILE 'BIOSIS'
           5328 PEPTIDYL
           6487 PROLYL
           1069 PEPTIDYL PROLYL
                  (PEPTIDYL(W)PROLYL)
          15328 ISOMERASE#
            996 PEPTIDYL PROLYL(3W)ISOMERASE#
            423 PPIASE#
           5528 PPI
L53        6612 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

FILE 'EMBASE'
           3390 "PEPTIDYL"
           4499 "PROLYL"
            807 PEPTIDYL PROLYL
                  ("PEPTIDYL"(W)"PROLYL")
           8932 ISOMERASE#
            749 PEPTIDYL PROLYL(3W)ISOMERASE#
            318 PPIASE#
           3251 PPI
L54        4057 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

FILE 'HCAPLUS'
           6944 PEPTIDYL
           8879 PROLYL
```

```
          1412 PEPTIDYL PROLYL
                 (PEPTIDYL(W)PROLYL)
         20028 ISOMERASE#
          1331 PEPTIDYL PROLYL(3W)ISOMERASE#
           580 PPIASE#
          4381 PPI
L55       5828 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

FILE 'NTIS'
            31 PEPTIDYL
            21 PROLYL
             4 PEPTIDYL PROLYL
                 (PEPTIDYL(W)PROLYL)
           104 ISOMERASE#
             3 PEPTIDYL PROLYL(3W)ISOMERASE#
             1 PPIASE#
           270 PPI
L56        273 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

FILE 'ESBIOBASE'
          2046 PEPTIDYL
          2213 PROLYL
           647 PEPTIDYL PROLYL
                 (PEPTIDYL(W)PROLYL)
          5695 ISOMERASE#
           602 PEPTIDYL PROLYL(3W)ISOMERASE#
           279 PPIASE#
          1558 PPI
L57       2211 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

FILE 'BIOTECHNO'
          1406 PEPTIDYL
          1423 PROLYL
           412 PEPTIDYL PROLYL
                 (PEPTIDYL(W)PROLYL)
          4091 ISOMERASE#
           386 PEPTIDYL PROLYL(3W)ISOMERASE#
           171 PPIASE#
           315 PPI
L58        731 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

FILE 'WPIDS'
           981 PEPTIDYL
          1223 PROLYL
           143 PEPTIDYL PROLYL
                 (PEPTIDYL(W)PROLYL)
          2153 ISOMERASE#
           141 PEPTIDYL PROLYL(3W)ISOMERASE#
            52 PPIASE#
           706 PPI
L59        867 PEPTIDYL PROLYL(3W)ISOMERASE# OR PPIASE# OR PPI

TOTAL FOR ALL FILES
L60      33216 PEPTIDYL PROLYL(3W) ISOMERASE# OR PPIASE# OR PPI

=> s l24 and l60
FILE 'MEDLINE'
L61           1 L13 AND L49

FILE 'SCISEARCH'
L62           1 L14 AND L50

FILE 'LIFESCI'
L63           2 L15 AND L51
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FILE 'BIOTECHDS'
L64           9 L16 AND L52

FILE 'BIOSIS'
L65           2 L17 AND L53

FILE 'EMBASE'
L66           1 L18 AND L54

FILE 'HCAPLUS'
L67          15 L19 AND L55

FILE 'NTIS'
L68           0 L20 AND L56

FILE 'ESBIOBASE'
L69           2 L21 AND L57

FILE 'BIOTECHNO'
L70           0 L22 AND L58

FILE 'WPIDS'
L71           8 L23 AND L59

TOTAL FOR ALL FILES
L72          41 L24 AND L60

=> s (l48 or l72) not 2004-2008/py
FILE 'MEDLINE'
       2589167 2004-2008/PY
                 (20040000-20089999/PY)
L73           0 (L37 OR L61) NOT 2004-2008/PY

FILE 'SCISEARCH'
       4718144 2004-2008/PY
                 (20040000-20089999/PY)
L74           0 (L38 OR L62) NOT 2004-2008/PY

FILE 'LIFESCI'
        536487 2004-2008/PY
L75           0 (L39 OR L63) NOT 2004-2008/PY

FILE 'BIOTECHDS'
        107093 2004-2008/PY
L76           2 (L40 OR L64) NOT 2004-2008/PY

FILE 'BIOSIS'
       2190807 2004-2008/PY
L77           0 (L41 OR L65) NOT 2004-2008/PY

FILE 'EMBASE'
       2266271 2004-2008/PY
L78           0 (L42 OR L66) NOT 2004-2008/PY

FILE 'HCAPLUS'
       5177725 2004-2008/PY
L79           3 (L43 OR L67) NOT 2004-2008/PY

FILE 'NTIS'
         63115 2004-2008/PY
L80           0 (L44 OR L68) NOT 2004-2008/PY

FILE 'ESBIOBASE'
       1321869 2004-2008/PY
L81           0 (L45 OR L69) NOT 2004-2008/PY
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FILE 'BIOTECHNO'
          586 2004-2008/PY
L82         0 (L46 OR L70) NOT 2004-2008/PY

FILE 'WPIDS'
       4380422 2004-2008/PY
L83         0 (L47 OR L71) NOT 2004-2008/PY

TOTAL FOR ALL FILES
L84         5 (L48 OR L72) NOT 2004-2008/PY

=> dup rem l84
PROCESSING COMPLETED FOR L84
L85          5 DUP REM L84 (0 DUPLICATES REMOVED)

=> d tot

L85   ANSWER 1 OF 5  BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
TI    New recombinant DNA molecule encoding a fusion protein comprising a
      sequence coding for a target polypeptide of a sequence coding for an FKBP
      chaperone, useful for immunizing animals or in producing a vaccine;
         vector-mediated recombinant protein gene transfer and expression in
         host cell for use in recombinant vaccine preparation
AU    SCHOLZ C; ANDRES H; FAATZ E; ENGEL A; SCHMITT U; BAZARSUREN A;
      SCHAARSCHMIDT P
AN    2003-11133  BIOTECHDS
PI    WO 2003000878 3 Jan 2003 equiv. to US 2003/0096352

L85   ANSWER 2 OF 5  BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
TI    Producing a soluble complex comprising an essentially insoluble target
      protein and a peptidyl-prolyl-isomerase
      class chaperone, useful in detecting antibodies to HIV, comprises
      solubilizing protein and the chaperone in a buffer;
         plasmid-mediated recombinant fusion proteiun gene transfer and
         expression in host cell for use in HIV virus diagnosis and recombinant
         vaccine
AU    SCHOLZ C; ANDRES H; FAATZ E; ENGEL A; SIZMANN D
AN    2003-11659  BIOTECHDS
PI    WO 2003000877 3 Jan 2003 equiv. to 2003/0176665

L85  ANSWER 3 OF 5  HCAPLUS  COPYRIGHT 2008 ACS on STN
TI   Recombinant protein expression as chaperonin fusion
     protein for X-ray crystal structure analysis
SO   Jpn. Kokai Tokkyo Koho, 8 pp.
     CODEN: JKXXAF
IN   Furuya, Masahiro; Hata, Junichi
AN   2003:734803  HCAPLUS
DN   139:257016
     PATENT NO.          KIND   DATE        APPLICATION NO.         DATE
     --------------      ----   --------    --------------------    --------
PI   JP 2003261597        A     20030919    JP 2002-353990          20021205

L85  ANSWER 4 OF 5  HCAPLUS  COPYRIGHT 2008 ACS on STN
TI   Method for refolding molecules of polypeptides containing Ig domains
SO   PCT Int. Appl., 35 pp.
     CODEN: PIXXD2
IN   Fersht, Alan Roy; Altamirano, Myriam Marlenne; Woolfson, Adrian; Milstein,
     Cesar
AN   2000:666754  HCAPLUS
DN   133:251276
     PATENT NO.          KIND   DATE        APPLICATION NO.         DATE
     --------------      ----   --------    --------------------    --------
PI   WO 2000055183        A1    20000921    WO 2000-GB987           20000316
        W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU,
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CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,
IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,
MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW,
AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE,
DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

CA 2364568	A1	20000921	CA 2000-2364568	20000316
EP 1161440	A1	20011212	EP 2000-911054	20000316

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO

L85 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2008 ACS on STN
TI The chaperonin cycle cannot substitute for prolyl isomerase activity, but GroEL alone promotes productive folding of a cyclophilin-sensitive substrate to a cyclophilin-resistant form
SO EMBO Journal (1997), 16(15), 4568-4578
CODEN: EMJODG; ISSN: 0261-4189
AU Von Ahsen, Oliver; Tropschug, Maximilian; Pfanner, Nikolaus; Rassow, Joachim
AN 1997:544620 HCAPLUS
DN 127:244366

=> d ab 1-5

L85 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
AB DERWENT ABSTRACT:
NOVELTY - A recombinant DNA molecule encoding a fusion protein, comprising at least one nucleotide sequence coding for a target polypeptide and upstream to at least one nucleotide sequence coding for a FKBP chaperone consisting of FkpA, SlyD or trigger factor, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an expression vector comprising operably linked a recombinant DNA molecule above; (2) a host cell transformed with the expression vector; (3) producing a fusion protein by culturing host cells of (2), expressing the fusion protein, and purifying the fusion protein; (4) a recombinantly produced fusion protein comprising at least one polypeptide sequence corresponding to a FKBP chaperone selected from of FkpA, SlyD and trigger factor, and at least one polypeptide sequence corresponding to a target peptide, and at least one peptidic linker sequence of 10-100 amino acids; and (5) a composition comprising a recombinantly produced fusion protein of (4), and a pharmaceutical excipient.

BIOTECHNOLOGY - Preferred DNA: The recombinant DNA molecule further comprises at least one nucleotide sequence coding for a peptidic linker of 10-100 amino acids located in between the sequence coding for the target polypeptide and the sequence coding for the FKBP chaperone. The recombinant DNA molecule comprises 1 or 2 nucleotide sequence coding for the FKBP chaperone. The 2 sequences coding for a FKBP chaperone are located upstream of the sequence coding for the target polypeptide, where one sequence coding for a PPI chaperone is located upstream of the target polypeptide and the other sequence coding for a PPI chaperone is located downstream of the sequence coding for the target peptide. The recombinant DNA molecule may also comprise 2 nucleic acid sequences coding for a linker polypeptide of 10-100 amino acids, where the 2 nucleic acid sequences coding for a linker of 10-100 amino acids are different or at least one of the linker sequences codes for a polypeptide linker comprising a proteolytic cleavage site. Preferred Fusion Protein: The fusion protein comprises 1 or 2 polypeptide sequences corresponding to the FKBP chaperone, where one of the two FKBP chaperones is located N-terminal and the other FKBP chaperone is located C-terminal to the target polypeptide. The peptidic linker sequences comprise a proteolytic cleavage site, and the target protein comprises a polypeptide

from an infectious organism. The polypeptide comprises at least one diagnostically relevant epitope of an infectious organism.

ACTIVITY - Immunostimulant. No supporting data provided.

MECHANISM OF ACTION - Vaccine.

USE - The recombinantly produced fusion protein is useful in providing an efficient expression system for recombinant proteins, for immunization of laboratory animals, in the production of a vaccine, or in an immunoassay (claimed).

EXAMPLE - The restriction site BamHI in the coding region of the mature E. coli FkpA was deleted using the QuikChange site-directed mutagenesis kit. HIV-1 gp41 (535-681)-His6 was cloned and expressed in a T7 promoter-based expression system. The gene fragment encoding amino acids 535-681 from HIV-1 envelope protein was PCR amplified from the T7-based expression vector, and was inserted into EckFpA(DELTABamHI)(GGGS)3 using BamHI and XhoI restriction sites. The codons for the glycine-serine-rich linker (GGS)3 between FkpA and e-gp41 were inserted with reverse primer for cloning of FkpA and with forward primer for cloning of e-gp41. The resulting construct was sequenced and found to encode the desired protein. E. coli harboring the expression plasmid were grown to an OD600 of 0.7, and cytosolic overexpression was induced by adding 1 mM of IPTG at a growth of temperature of 37degreesC. Four hours after induction, cells were harvested by centrifugation, and bacterial pellet was resuspended in 50 mM sodium phosphate, 5 mM imidazole and stirred at room temperature for complete lysis. After repeated centrifugation, supernatant was filtered and applied to a Ni-NTA-column, and unspecifically bound proteins were removed. Bound target protein was eluted with 50 mM sodium phosphate and collected in 4 ml fractions. After solubilization, material was transferred into physiological buffer conditions by dialysis. Refolding of the HIV-1 gp41 part of the fusion protein (ectodomain) was induced by removing guHCl from the eluted protein by dialysis against 50 mM sodium phosphate. Analysis of recombinantly produced FkpA using near UV CD showed that FkpA was essentially unstructured under the same conditions. Refolding of gp41-FkpA by dialysis resulted in a readily soluble protein complex comprising the covalently linked gp41 and FkpA protein domains. (18 pages)

L85 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

AB DERWENT ABSTRACT:

NOVELTY - Producing a soluble complex comprising a target protein which is essentially insoluble and a peptidyl-prolyl-isomerase class chaperone comprises mixing the protein and the chaperone in a buffer where both the protein and the chaperone are solubilized, and adjusting the buffer to physiological conditions where the protein-chaperone complex formed is soluble.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a soluble complex comprising a retroviral surface glycoprotein and a peptidyl prolyl isomerase chaperone, where the retroviral surface glycoprotein and the peptidyl prolyl isomerase chaperone are covalently linked; (2) a composition of reagents comprising the soluble complex; (3) a method of detecting at least one antibody to an envelope virus surface glycoprotein in a sample by contacting the sample and a composition containing a complex of the surface glycoprotein and a peptidyl prolyl isomerase chaperone, and detecting bound antibodies; (4) an immunoassay according to the double antigen bridge concept, comprising a first antigen consisting of a first chaperone-antigen complex, and a second antigen consisting of a second chaperone-antigen complex; (5) a method of eliciting an immune response by injecting into the subject a vaccine comprising a soluble retroviral surface glycoprotein-chaperone complex to elicit antibodies that bind the retroviral surface glycoprotein; and (6) a method of inhibiting virus entry into a cell by administering to a cell a soluble complex above.

BIOTECHNOLOGY - Preferred Method: The physiological buffer comprises a buffer compound in a concentration of 10-200 mM and a total salt

concentration of 20-500 mM. The protein and the peptidyl prolyl isomerase are produced recombinantly. The protein is an amyloidogenic protein, such as a retroviral surface glycoprotein, an HIV-2 gp36, or HIV-1 gp41. The peptidyl prolyl isomerase is a binding-competent fragment of the peptidyl prolyl isomerase or an FKBP chaperone selected from SlyD, FkpA and a trigger factor. The soluble complex is a retroviral surface glycoprotein-chaperone complex. The complex may be produced by solubilizing a retroviral surface glycoprotein covalently linked to a peptidyl prolyl isomerase in a buffer where the retroviral surface glycoprotein is solubilized, and adjusting the buffer to physiological conditions where the retroviral surface glycoprotein-chaperone complex is soluble. In detecting at least one antibody to an envelope virus surface glycoprotein, the sample is contacted under conditions allowing the binding of the antibodies to the surface glycoprotein and the presence of bound antibodies indicates the presence of anti-viral antibodies in the sample. In the immunoassay according to the double antigen bridge concept, the first chaperone and the second chaperone are different molecules derived from one species or from different species. The first and/or second chaperone is derived from a thermophilic bacteria. The first antigen complex comprises a solid phase binding group and the second antigen complex comprises a marker group. Inhibiting virus entry comprises inhibiting membrane fusion. The physiological buffer comprises a buffer compound in a concentration of 10-200 mM and a total salt concentration of 20-500 mM. The protein and the peptidyl prolyl isomerase are produced recombinantly. The protein is an amyloidogenic protein, preferably a retroviral surface amyloidogenic glycoprotein, an HIV-2 gp36 or an HIV-1 gp41. The peptidyl prolyl isomerase is a binding-competent fragment of the peptidyl prolyl isomerase. Preferred Complex: The retroviral surface glycoprotein and the peptidyl prolyl isomerase chaperone are covalently linked, specifically chemically coupled or recombinantly linked. The recombinant linkage comprises a peptide linker of at least 10-50 amino acids.

ACTIVITY - Immunostimulant; Anti-HIV. No biological data given.

MECHANISM OF ACTION - Vaccine. No biological data given.

USE - The method is useful for producing soluble retroviral surface glycoprotein-chaperone complex. The chaperone-antigen complex is useful in detecting antibodies to HIV in immunoassays, specifically according to the double antigen bridge concept, or as an immunogen. Compositions comprising gp41-chaperone complex and/or a gp36-chaperone complex may be used to prevent HIV entry and spread within the host organism, and for eliciting an immune response in a mammal.

ADMINISTRATION - Typical dose is 1 fg-1 mg, preferably 100 ng-50 micrograms per kg body weight. Administration can be intradermal, subcutaneous, intramuscular, intraperitoneal, inhalation, topical, by suppository, or using a transdermal patch.

EXAMPLE - The restriction site BamHI in the coding region of the mature E. coli FkpA was deleted using the QuikChange site-directed mutagenesis kit. The gene fragment encoding amino acids 535-681 from HIV-1 envelope protein was PCR amplified, and was inserted into EckFpA(DELTABamHI)(GGGS)3 using BamHI and XhoI restriction sites. The codons for the glysine-serine-rich linker (GGS)3 between FkpA and e-gp41 were inserted with reverse primer for cloning of FkpA and with forward primer for cloning of e-gp41. The resulting construct was sequenced and found to encode the desired protein. E. coli harboring the expression plasmid were grown to an OD600 of 0.7, and cytosolic overexpression was induced by adding 1 mM of IPTG at a growth of temperature of 37 degrees Centigrade. Four hours after induction, cells were harvested by centrifugation, and bacterial pellet was resuspended in 50 mM sodium phosphate, 5 mM imidazole and stirred at room temperature for complete lysis. After repeated centrifugation, supernatant was filtered and applied to a Ni-NTA-column, and unspecifically bound proteins were

removed. Bound target protein was eluted with 50 mM sodium phosphate and collected in 4 ml fractions. Unfolded gp41-FkpA polypeptide was applied on a Superdex 200 gel filtration column equilibrated with 20 mM sodium phosphate, 50 mM NaCl, 1 mM EDTA. FkpA-gp41 elutes essentially in 3 main fractions: as a high molecular associate, as an apparent hexamer species, and as an apparent trimer species. The apparent trimer fraction was concentrated and assessed for its tertiary structure in a near ultra-violet (UV) CD measurement. Gp41 displayed tertiary structure at neutral pH and was evidently solubilized by the covalently bound chaperone. The chaperone FkpA seemed to accept the native-like structured ectodomain gp41 as substrate and to solubilize this hard-to-fold protein at a neutral working pH.(36 pages)

L85 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2008 ACS on STN
AB Provided is a method for crystallization of any desired protein by recombinant expression as fusion protein with chaperonin subunit(s) for X-ray crystal structure anal. A target protein is either ligated to a chaperonin subunit via peptide bond or incorporated into the tertiary structure of the ring formed by chaperonin subunits. The target protein may be a membrane protein or nuclear hormone receptor. Expression of human cyclophilin (cyclosporin-binding protein hCyp) as fusion protein with Thermococcus chaperonin α subunit and expression of GFP as fusion protein with E. coli GroEL, and X-ray crystal structure study, are described.

L85 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2008 ACS on STN
AB A method is provided for promoting the folding of a polypeptide comprising at least one Ig domain which method comprises contacting the polypeptide with a mol. chaperone and a foldase.

L85 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2008 ACS on STN
AB The chaperonin GroEL and the peptidyl-prolyl cis-trans isomerase cyclophilin are major representatives of two distinct cellular systems that help proteins to adopt their native three-dimensional structure: mol. chaperones and folding catalysts. Little is known about whether and how these proteins cooperate in protein folding. In this study, we have examined the action of GroEL and cyclophilin on a substrate protein in two distinct prolyl isomerization states. Our results indicate that: (i) GroEL binds the same substrate in different prolyl isomerization states. (Ii) GroEL-ES does not promote prolyl isomerizations, but even retards isomerizations. (Iii) Cyclophilin cannot promote the correct isomerization of prolyl bonds of a GroEL-bound substrate, but acts sequentially after release of the substrate from GroEL. (I.v.) A denatured substrate with all-native prolyl bonds is delayed in folding by cyclophilin due to isomerization to non-native prolyl bonds; a substrate that has proceeded in folding beyond a stage where it can be bound by GroEL is still sensitive to cyclophilin. (V) If a denatured cyclophilin-sensitive substrate is first bound to GroEL, however, productive folding to a cyclophilin-resistant form can be promoted, even without GroES. We conclude that GroEL and cyclophilin act sequentially and exert complementary functions in protein folding.

=> fil .becpat

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	45.89	46.10
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-2.40	-2.40

FILES 'BIOTECHDS, HCAPLUS, WPIDS' ENTERED AT 16:47:12 ON 09 JAN 2008

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3 FILES IN THE FILE LIST

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                 (PRY<=2003)
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L86           2 (L40 OR L64) AND WO/PC AND PRY<=2003 AND PY>=2004

FILE 'HCAPLUS'
        286232 WO/PC
        800112 PRY<=2003
       4774876 PY>=2004
L87           5 (L43 OR L67) AND WO/PC AND PRY<=2003 AND PY>=2004

FILE 'WPIDS'
        507817 WO/PC
       1502382 PRY<=2003
       3288202 PY>=2004
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L90  ANSWER 1 OF 7  HCAPLUS  COPYRIGHT 2008 ACS on STN
TI   Versatile platform for nanotechnology based on circular permutations of
     chaperonin TF55β from Sulfolobus shibatae
SO   U.S. Pat. Appl. Publ., 151 pp., Cont.-in-part of U.S. Ser. No. 494,853.
     CODEN: USXXCO
IN   Paavola, Chad D.; Trent, Jonathan D.; Chan, Suzanne L.; Li, Yi-Fen;
     McMillan, R. Andrew; Kagawa, Hiromi
AN   2006:367306  HCAPLUS
DN   144:407655
     PATENT NO.          KIND   DATE        APPLICATION NO.         DATE
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PI   US 2006084792        A1    20060420    US 2005-194991          20050801 <--
     WO 2003080796        A2    20031002    WO 2002-US35889         20021108 <--
     WO 2003080796        A9    20040429
     WO 2003080796        A3    20051222
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
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             CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
     US 2005130258        A1    20050616    US 2004-494853          20040506 <--

L90  ANSWER 2 OF 7  BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
TI   Fusion protein useful for preparing target protein such as
     immunoglobulin, has molecular chaperone connected to terminal of
     intercalated protein having activity of cleaving peptide bond, and target
     protein;
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isolation of a recombinant fusion protein having intein and green fluorescent protein useful for production of immunoglobulin
AU TOGI A; FURUTANI M
AN 2005-00070 BIOTECHDS
PI WO 2004096860 11 Nov 2004

L90 ANSWER 3 OF 7 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
TI Novel immunogen comprising fused protein of full-length or part of antigen (serotonin 5 HT1aR) and folding factor (peptidylprolyl isomerase) or its subunit bonded together through peptide bonds, useful for inducing immune response;
vector-mediated immunogen gene transfer and expression in host cell for recombinant vaccine
AU IZUMOTO Y; HATA J; IDENO A; FURUTANI M
AN 2004-25618 BIOTECHDS
PI WO 2004092221 28 Oct 2004

L90 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN DUPLICATE 3
TI Production of recombinant antibodies as chaperonin fusion protein
SO Jpn. Kokai Tokkyo Koho, 23 pp.
CODEN: JKXXAF
IN Ideno, Akira; Hata, Junichi; Togi, Akiko; Furuya, Masahiro
AN 2004:216907 HCAPLUS
DN 140:265615

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 2004081199	A	20040318	JP 2003-124351	20030428 <--
WO 2004097018	A1	20041111	WO 2004-JP1839	20040218 <--

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L90 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN
TI Chaperonin-target protein complex, method of producing the same, method of stabilizing target protein, method of immobilizing target protein, method of analyzing the structure of target protein, sustained-release preparation and method of producing antibody against target protein
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
IN Ideno, Akira; Hata, Jun-ichi; Togi, Akiko; Furutani, Masahiro
AN 2004:965292 HCAPLUS
DN 141:428008

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2004096859	A1	20041111	WO 2004-JP6189	20040428 <--

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
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AU 2004234282	A1	20041111	AU 2004-234282	20040428 <--

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     CA 2522263          A1    20041111    CA 2004-2522263        20040428 <--
     EP 1619208          A1    20060125    EP 2004-730054         20040428 <--
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            IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL, SK, HR
     US 2007059794       A1    20070315    US 2005-554747         20051028 <--

L90  ANSWER 6 OF 7  HCAPLUS  COPYRIGHT 2008 ACS on STN
TI   Soluble complexes of amyloid β (Aβ) target proteins and
     peptidyl prolyl isomerase (Ppplase)
     chaperones, their preparation and use in diagnosis of Alzheimer's disease and
     as immunogens
SO   PCT Int. Appl., 39 pp.
     CODEN: PIXXD2
IN   Faatz, Elke; Scholz, Christian; Schaarschmidt, Peter
AN   2004:546522  HCAPLUS
DN   141:102770
     PATENT NO.          KIND   DATE        APPLICATION NO.        DATE
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PI   WO 2004056855       A1     20040708    WO 2002-EP14631        20021220 <--
     WO 2004056855       A9     20050714
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     CA 2510717          A1     20040708    CA 2002-2510717        20021220 <--
     AU 2002363891       A1     20040714    AU 2002-363891         20021220 <--
     EP 1578793          A1     20050928    EP 2002-798355         20021220 <--
     EP 1578793          B1     20071003
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     JP 2006522583       T      20061005    JP 2004-561108         20021220 <--
     AT 374784           T      20071015    AT 2002-798355         20021220 <--
     US 2005112720       A1     20050526    US 2003-443654         20030522 <--
     US 7094884          B2     20060822

L90  ANSWER 7 OF 7  HCAPLUS  COPYRIGHT 2008 ACS on STN
TI   Expression vector containing chaperonin PPIase for improvement
     of the expression efficiency for foreign proteins
SO   PCT Int. Appl., 73 pp.
     CODEN: PIXXD2
IN   Ideno, Akira; Maruyama, Tadashi; Furutani, Masahiro
AN   2004:3039  HCAPLUS
DN   140:72133
     PATENT NO.          KIND   DATE        APPLICATION NO.        DATE
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PI   WO 2004001041       A1     20031231    WO 2003-JP8020         20030625 <--
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            BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
     CA 2490384          A1     20031231    CA 2003-2490384        20030625 <--
     AU 2003243969       A1     20040106    AU 2003-243969         20030625 <--
     EP 1516928          A1     20050323    EP 2003-733573         20030625 <--
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R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK
US 2005130259 A1 20050616 US 2004-511098 20041014 <--

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L90 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN
AB The present invention provides chaperonin polypeptides which are modified to include N-terminal and C-terminal ends that are relocated from the central pore region to various different positions in the polypeptide which are located on the exterior of the folded modified chaperonin variant. In the modified chaperonin, the naturally-occurring N-terminal and C-terminal ends are joined together directly or with an intervening linker peptide sequence. The relocated N-terminal or C-terminal ends can be covalently joined to, or bound with another mol. such as a nucleic acid mol., a lipid, a carbohydrate, a second polypeptide, or a nanoparticle. The modified chaperonin variants can assemble into double-ringed chaperonin structures. Further, the chaperonin structures can organize into higher order structures such as nanofilaments or nanoarrays which can be used to produce nanodevices and nanocoatings. In particular, the invention provides modified variants of Sulfolobus shibatae chaperonin TF55β. The sequences of the S. shibatae chaperonin TF55β modified variants are provided.

L90 ANSWER 2 OF 7 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
AB DERWENT ABSTRACT:
NOVELTY - A fusion protein (I) comprises a molecular chaperone or its subunit connected to a terminal of intercalated protein which has an activity of cleaving a peptide bond, and a target protein connected to other terminal by a peptide bond.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated gene (II) encoding (I); (2) an isolated gene (III) encoding a partial sequence of intein protein; (3) an expression vector (IV) comprising (II) or (III); (4) a transformed host comprising (IV); and (5) preparing a target protein, involves: (a) connecting a molecular chaperone or its subunit to a terminal of partial sequence of intein protein by a peptide bond, producing first fusion protein which connected a target protein first precursor to the other terminal by a peptide bond, connecting a molecular chaperone or its subunit to a terminal of the remaining partial sequence of intein protein by a peptide bond, producing a second fusion protein which connected the target protein second precursor to the other terminal by a peptide bond, cutting a target protein first precursor from the first fusion protein obtained in the above process by the cleavage action of the partial sequence of intein protein and the remaining partial sequence of the intein protein, further cutting the target protein second precursor from the second fusion protein obtained by the above mentioned process, and assembling the target protein first precursor and target protein second precursor obtained by the above process, by the assembly function of partial sequence of intein protein and the remaining partial sequence of the intein protein; or (b) connecting a partial sequence of intein protein to a terminal of molecular chaperone, and connecting the remaining partial sequence of the intein protein to the other terminal by a peptide bond, further connecting a target protein first precursor to the partial sequence of intein protein by a peptide bond, and from a fusion protein having the target protein first precursor and the target protein second precursor connected by a peptide bond, cutting target protein first precursor and target protein second precursor by the cleavage action of the partial sequence of intein protein and remaining partial sequence of intein protein, and further assembling the target protein first precursor and target protein second precursor by the assembly function of the partial sequence of intein protein and remaining partial sequence of intein protein.

BIOTECHNOLOGY - Preferred Protein: In (I), the molecular chaperone

is a chaperonin containing a number of chaperonin subunits. The intercalated protein having peptide bond cleaving activity is connected to an individual chaperonin subunit, or a chaperonin subunit connecting material, where the 2-10 chaperonin subunits connected the chaperonin subunit connecting material in series through a peptide bond. The intercalated protein having a peptide bond cleaving activity is connected to the region of one or more of an individual chaperonin subunit or individual N-terminal of chaperonin subunit connecting material; the C-terminal of an individual chaperonin subunit or an individual chaperonin subunit connecting material; or the connecting portion of the chaperonin subunits of a chaperonin subunit connecting material. The ratio of the number of chaperonin subunit and target protein is 1:1-10:1. The molecular chaperone is peptidyl prolyl cis-trans isomerase, and is derived from bacteria, archaebacterium or eukaryote. The intercalated protein having peptide bond cleaving activity is connected to N-terminal and/or the C-terminal of peptidyl prolyl cis-trans isomerase. The intercalated protein having a peptide bond cleaving activity is intein or a partial sequence of intein, where intein cleaves N-terminal and does not cleave a C-terminal, or cleaves C-terminal and does not cleave N-terminal. The partial sequence of intein consists of 20-120 amino acids of C-terminal of intein. The intein is derived from Synechocystis sp., Mycobacterium xenopi, Saccharomyces cerevisiae or Halobacterium sp.. The partial sequence of intein has a fully defined sequence of 154 amino acids (S1) as given in the specification or (S1) in which one or more amino acids deleted, substituted, added or inserted, where the protein has peptide cleaving activity. The partial sequence of intein has an amino acid sequence, exhibiting at least 50% or more homology with (S1), where the protein has peptide cleaving activity. The intercalated protein having peptide cleaving activity is protease.

USE - (I) is useful for preparing a target protein, which involves: (a) cutting out the target protein from (I) by the action of the intercalated protein having a peptide cleaving activity; or (b) preparing (I), inducing peptide cleaving activity of the intercalated protein contained in (I) prepared in the above process, cleaving a portion of (I) by the action of the intercalated protein whose peptide cleaving activity is induced in the above process, and cutting out and separating the target protein from (I). The peptide cleaving activity is induced by exposing (I) to a temperature of 20-37degreesC, pH of 6-8, and by adding thiol. The method involves producing an expression vector having a gene that encodes (I), introducing the expression vector obtained by above process into a host and expressing (I), and cutting out the target protein from (I) expressed in the above process. The method involves integrating a gene encoding fusion protein in two types of different plasmids that can be co-existed and expressed within the same host, and producing two types of expression vectors, introducing the two types of expression vectors obtained in the above process into the same host and expressing (I), and cutting out the target protein by the action of intercalated protein having peptide bond cleaving activity from (I). The method involves integrating a gene that encodes (I) in one side of two types of different plasmids that can be co-existed and express within the same host, integrating a gene that encodes only the molecular chaperone on the other side, and producing two types of expression vectors; introducing the two types of expression vectors obtained by the above process into the same host; expressing (I) and the molecular chaperone; and cutting out the target protein by the action of intercalated protein having peptide cleaving activity, from (I) obtained by the above process. The host is bacteria, yeast, animal cell, plant cell, insect cell, an animal, plant or an insect. The method involves making (I) express within the host as mentioned above. The method is carried out with a non-cell translation system. The molecular chaperone is chaperonin. The 5-10 chaperonin subunits are gathered in ring shape to form a chaperonin ring, where the target protein is accommodated within the chaperonin ring (all claimed). (I) is useful in preparing immunoglobulin.

ADVANTAGE - (I) enables to prepare a target protein, efficiently.

(I) is expressed more easily by gene modification technology. The intercalated protein having peptide bond cleaving activity enables to cut the target protein safely and conveniently.

EXAMPLE - Expression of fusion protein was carried out as follows. A plasmid pETTPPIaseI having T7 promoter, peptidyl prolyl cis-trans isomerase gene (TPPIase), Synechocystis sp. intein gene (SspI) and His gene was prepared. The gene encoding green fluorescent protein (GFP) was introduced between SpeI site and HpaI site. Thus, the expression vector pETTPPIase-GFP capable of synthesizing fusion protein of SspI and GFP was built. The obtained expression vector was introduced into Escherichia coli BL21 (DE3) strain. The transformed organism was cultivated in YT culture medium containing carbenicillin at 25degreesC for 24 hours. Thus, a fusion protein comprising TPPIase, SspI and GFP, in the culture was obtained. (76 pages)

L90 ANSWER 3 OF 7 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

AB DERWENT ABSTRACT:

NOVELTY - An immunogen (I) for inducing an immune response against a desired antigen protein, comprises a fused protein in which the full-length or a part of a desired antigen protein and a folding factor or its subunit are bonded together through one or more peptide bonds, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) producing (I), involves translating a fusion gene that encodes a full-length or a part of a desired antigen protein and a folding factor or its subunit; and (2) composition (II) for immunological use, formed by mixing (I) and an adjuvant.

BIOTECHNOLOGY - Preferred Immunogen: In (I), the folding factor is chaperonine, which comprises number of chaperonine subunits. One or all parts of the chaperonine subunits are mutually connected by peptide bonds. The desired antigen protein is connected to the N- or C-terminal of the chaperonine subunits. The antigen protein is connected among the chaperonine subunits. (I) has a protease cleavage site between the chaperonine subunits and the antigenic protein. The chaperonine subunits are derived from bacteria, preferably archaebacterium or from eukaryotes. The antigen protein is contained inside the chaperonine ring formed by the chaperonine subunits. The chaperonine ring comprises 5-10 chaperonine subunits. The folding factor includes chaperonine and PPIase. The PPIase is derived from Escherichia coli or archaebacterium. The antigenic protein is serotonin 5 HT1aR. The fusion protein comprises full length serotonin 5 HT1aR or a partial peptide comprising 6 or more residues of serotonin 5 HT1aR.

ACTIVITY - Immunostimulant.

MECHANISM OF ACTION - None given.

USE - (I) or (II) is useful for producing an antibody specific to the antigen protein, which involves immunizing an animal (except human) using (I) or (II) and extracting the antibody from the animal (claimed).

ADVANTAGE - (I) prevents the antigen protein from quick degradation in the blood of an animal. (I) provides effective immune response against a desired antigenic protein and enables reliable production of an antibody against the desired antigenic protein.

EXAMPLE - Immunogen comprising Escherichia coli derived chaperonine GroEL and an antigenic protein was produced as follows. Vector capable of expressing the fusion protein of GroEL and antigenic protein was assemble. The antigenic protein is full length serotonin 5 HT1aR or a partial peptide comprising 6 or more residues of serotonin 5 HT1aR. The vector was introduced into a suitable host and the fusion protein comprising 7 subunits of GroEL and recombinant serotonin 5 HT1aR receptor was produced. The stereostructure of the obtained fusion protein was examined. The structure analysis showed that the chaperonine subunits formed a ring structure inside which the antigenic protein was contained. The serotonin 5 HT1aR receptor was protected by GroEL. The immunogenicity of the fusion protein (immunogen) was evaluated. The fusion protein was admixed with incomplete Freund's

adjuvant and a composition for immunological use was obtained. The composition was administered (subcutaneously) to a rabbit and antibody titer against serotonin 5 HT1aR receptor was measured. The results showed that the immunogen was efficient in inducing immune response against serotonin 5 HT1aR receptor (antigenic protein). (59 pages)

L90 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN DUPLICATE 3
AB Provided is a method of antibody production by expressing heavy chain and/or light chain as fusion protein with chaperonin and reconstituting after protease cleavage from the fusion partner. 2-10 Chaperonin subunits are linked via peptide bond and antibody heavy chain and/or light chain are linked to its N-terminal, C-terminal, or linkage between subunits. A preferred form is where 2 chaperonin rings comprising 5-10 chaperonin subunits form a noncovalent two-layer structure via the ring surface and antibody heavy chain and/or light chain are enclosed within. Humanized antibodies or chimeric antibodies may be produced. Fusion proteins of human anti-hepatitis B surface antigen (HBs) antibody heavy chain and of light chain with Thermococcus KS-1 chaperonin β subunit were produced in E. coli. Binding ability of the antibody reconstituted from heavy chain and light chain was higher than that of the heavy chain alone.

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AB It is intended to provide a complex of chaperonin and a target protein by which the target protein can be handled more easily, and a method of producing the same; and a method of stabilizing the target protein, a method of immobilizing the target protein, a method of analyzing the structure of the target protein, a sustained-release agent and a method of producing an antibody against the target protein, each using the chaperonin-target protein complex. The above-described chaperonin-target protein complex contains a fused protein in which an affinity tag is attached to chaperonin subunits via a peptide bond, and a target protein to which the affinity tag shows a specific affinity. Owing to the specific affinity, the target protein is bonded to the affinity tag, thus forming a chaperonin ring structure consisting of a plural number of chaperonin subunits. Using to the chaperonin-target protein complex, the target protein can be stabilized and surely immobilized on a support without causing any change in its stereostructure. Thus, IgG was incubated with a chaperonin β-subunit-protein A fusion protein produced in E. coli to obtain a chaperonin -IgG complex.

L90 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2008 ACS on STN
AB The disclosed invention relates to the production of a soluble Aβ-chaperone complex and wherein the chaperone is a member of the Ppplase family and the advantageous use of such chaperone-Aβ complex, especially in the detection of Aβ in an immunoassay, as well as its use as an immunogen. In a presented experiment an expression vector was constructed comprising E. coli-derived SlyD chaperone as the fusion partner and Aβ(1-42) as target protein. The methods described facilitate the convenient recombinant production of an Aβ in a soluble form and in high amts. (yield >20 mg fusion protein/g wet weight of E. coli cell mass).

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